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Applicant: MADSEN, Lars Siim et al.
Appl. No.: 10/518,554
Filed: December 22, 2004
Conf.: 8395
Art Unit: 1626
Examiner: Stockton, Laura Lynne
Title: NOVEL BENZIMIDAZOL-2-ONE DERIVATIVES AND THEIR USE

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR 1.132

I, *Morten Grunnet*, do hereby declare that:

I am a citizen of Denmark, residing at Strandøre 12, DK-2100 Copenhagen Ø, Denmark.

I received a Master in Biology and a PhD in Medicine in 1998 and 2002, respectively.

I became employed at NeuroSearch A/S as Research Scientist in 2003. My scientific experience is mainly on medicinal chemistry in the area of voltage and ligand gated ion channels.

I am the author or co-author of 45 scientific papers and patent applications in the field of membrane biology with special emphasis on ion channels.

The subject matter of the invention of the present application is not my invention, but rather the invention of the inventors named therein, namely *MADSEN, Lars Siim et al.* However, I do consider myself, based upon my aforesaid qualifications and experience, to be one skilled in the art to which this application is directed.

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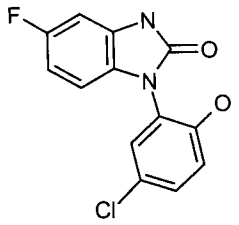
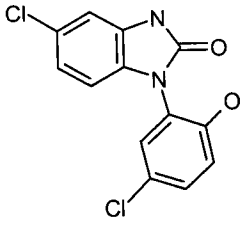
I have been asked to study the present patent application, Serial No. 10/518,554, filed December 22, 2004, as well as the Official Actions in the present patent application dated June 26, 2006, and December 7, 2006.

I have been asked to do a comparison of 1,3-dihydro-1-(5-chloro-2-hydroxyphenyl)-5-fluoro-2H-benzimidazol-2-one of the prior art, i.e. Ex. 10 of EP 477819 (Compound A), and 1-(5-chloro-2-hydroxyphenyl)-5-chloro-1,3-dihydro-2H-benzimidazo-2-one of the invention (Compound B):



Compound A



Compound B

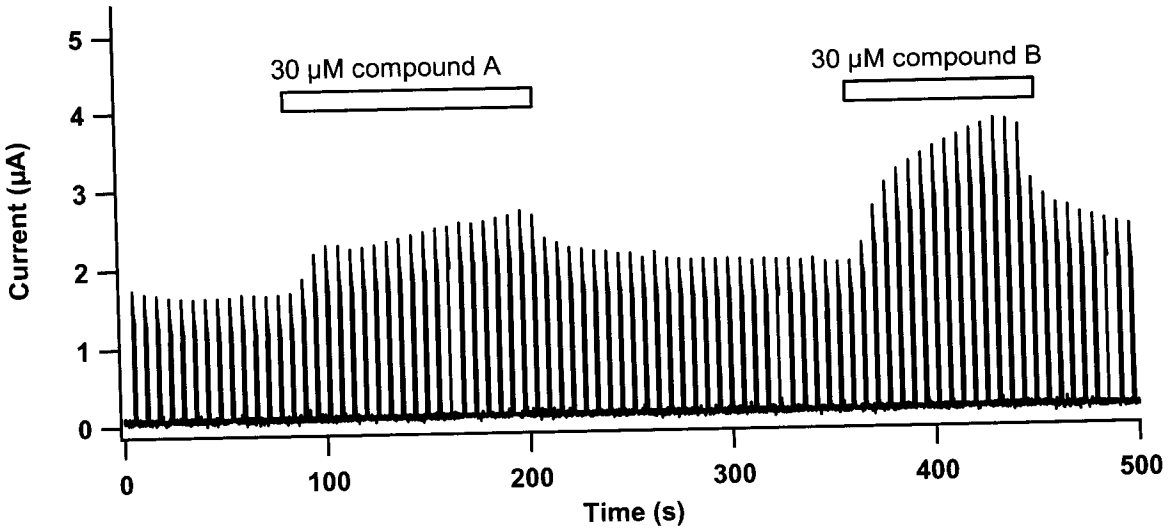
First BK channels were expressed in *Xenopus laevis* oocytes. In vitro transcription of cRNA encoding the human BK channel (KCNMA1, hslo, MaxiK) was performed using Ambion T7 m-Message Machine kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. mRNA was purified using the Ambion MEGAclear kit (Ambion, Austin, TX, USA) according to the manufactures instruction and dissolved in TE buffer to approximate concentrations of 0.1 µg/µl. For proof of purity and integrity, mRNA was inspected by gel electrophoresis and concentrations were determined photometrically. mRNA was stored at -80°C until injection. BK channels were heterologously expressed in oocytes from the frog *Xenopus laevis.* Oocytes were collected under anaesthesia (Tricain 2 g/l, Sigma A-5040) at guidelines approved by the Danish National Committee for Animal Studies. Before injection of 50 nl mRNA (approximately 5 ng), oocytes were kept for 24 hours at 19°C in Kulori medium consisting of (in mM) 90 NaCl, 1 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 5 HEPES, pH 7.4 with NaOH. Injection of mRNA was accomplished using a Nanoject microinjector from Drummond (Drummond Scientific, Broomall, PA, USA). Oocytes were kept at 19°C for 1-3 days before measurements were performed.

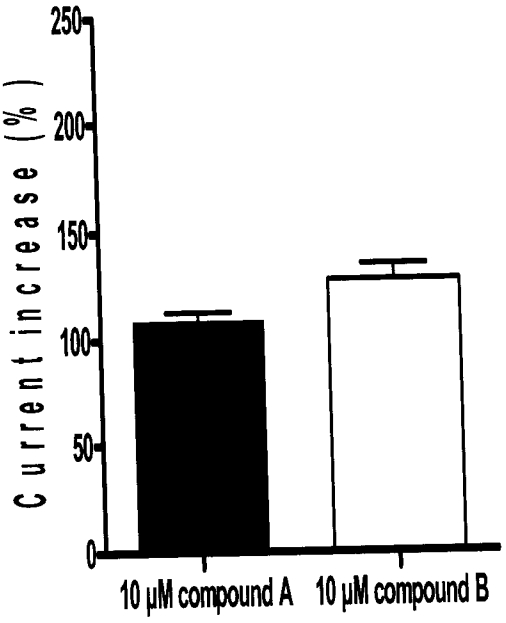
Current through BK channels expressed in *Xenopus laevis* oocytes was monitored using a two-electrode voltage-clamp amplifier (Dagan CA-1B, Minneapolis, MN, USA). Electrodes were pulled from borosilicate glass capillaries on a horizontal patch electrode puller (DMZ universal puller, Zeitz instruments, München, Germany) and had tip resistance between 0.5 and 2.5 MΩ when filled with 2 M KCl. During the experiments oocytes were placed in a small chamber (volume: 200 µl) connected to a continuous flow system (flow: 3 ml/min.). BK channels were activated by membrane depolarization and channel activity was measured in Kulori solution consisting of (in mM) 87 NaCl, 4 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 5 HEPES, pH 7.4 with NaOH. All experiments were performed at room temperature. The condition of each single oocyte was controlled before measurements by recording membrane potentials. Only oocytes with membrane potentials below -30 mV were used for current recordings. Tested compounds were dissolved in DMSO and diluted 1000 times in Kulori medium. At this concentration, no effect of the applied vehicle was observed (data not shown).

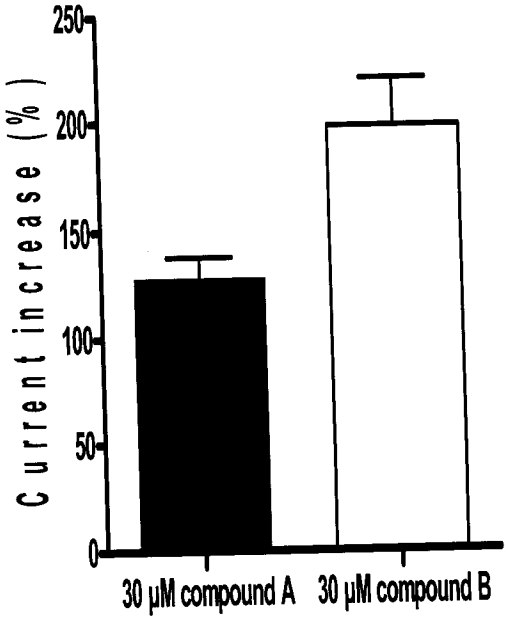
BK channels expressed in *Xenopus laevis* oocytes were activated by repeated depolarizing and repolarising pulses from -80 mV to +20 mV lasting for 5 and 1 respectively. Current increase was examined as peak current increase at depolarized potentials. Current values obtained at 10 µM

were for Compound A 109 ± 8 % and for Compound B 128 ± 14. The corresponding values obtained for 30 µM was 129 ± 20 and 198 ± 45, respectively.

For both concentrations Compound B increased BK current significantly better than did Compound A (p values 0.01 and 0.02, respectively), which difference was not obvious to the person skilled in the art. Deviations are indicated as S.D.







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I hereby declare that, to my own knowledge, all statements made herein are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful and false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Executed by me at Ballerup, Denmark, this 30 day of January 2007.

Morten Grunnet